The University of Chicago

# THE CONDITIONS NECESSARY FOR THE GERMINATION OF THE SEEDS OF SOME PLANTS NATIVE TO THE CHICAGO REGION

A PART OF A DISSERTATION SUBMITTED TO
THE GRADUATE FACULTY IN CANDIDACY FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF BOTANY
1930

By
MAY BURUNJIK

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## INTRODUCTION

The purpose of this investigation was to find the conditions necessary for the germination of some of the plants native to the Chicago region and to note the time required by the later stages of their development. Such a study would form a basis for the more intelligent judgment of the possibility of cultivating these plants from seed and of thereby aiding their preservation. It is a continuation of the work done by Miss Harriet George at this laboratory.

The earlier literature has been summarized by George (7). More recent work will be mentioned briefly here. Knudson (13, 14) worked on orchid seeds to find what was necessary for a high percentage of germination. A later paper by Adams (1) is on the germination of the seeds of some plants with fleshy fruits, evidently from the eastern United States. Both wild and cultivated plants were included. Ricker (20) of the Wild Flower Preservation Society gives some general notes on the germination and cultivation of native plants. Johnson reports the germination of seeds of Gentiana crinita (9).

## METHODS

The method used in testing germination was as follows. The seeds were thoroughly washed and freed from any fleshy material around them. They were then air dried and stored in corked glass bottles at room temperature. Directly before being used they were sterilized. Fifteen minutes in sterile water, thirty in a 0.25 Uspulin solution, and another fifteen in sterile water was the schedule used. The seeds were then put into Petri dishes which had previously been fitted with cellucotton and autoclaved at 15 pounds pressure for half an hour. Distilled water was used

for moistening the seeds.

As Miss George observed, a coating of paraffin keeps the labels relatively free from mold. The writer found it advantageous to use pieces of plain paper which were fastened to the glass cover only by the paraffin rather than gummed labels. Even when paraffined, the gum furnishes an excellent medium for mold.

"Minneapolis" germinators, electrically heated, and cooled by a water jacket were used for the 25°C. and 20°C. temperatures. The moist atmosphere maintained in these germinators keeps the dishes from drying rapidly and reduces the possibility of contamination in moistening the cellucotton.

The dishes were checked about once a week for germination and for contamination. If the mold was not too far advanced, all the seeds of the dish were sterilized as before and put into a clean dish. If the seeds were badly molded, they were discarded. Without this constant check on the mold, the experiments could not have been run for as long a period as they were, and long periods of dormancy could not have been ascertained.

When the coats were removed, the seeds were previously sterilized and the decorticated seeds simply washed in sterile water. This method was not altogether satisfactory, but it was believed that the Uspulin treatment might be injurious to the naked seed.

The sugar solutions used were sterilized in the autoclave at 12 pounds pressure for thirty minutes. The soil was likewise sterilized in the autoclave at 15 pounds pressure. It was an ordinary greenhouse mixture of loam and organic material and gave a neutral reaction with a La Motte tester. The acid peat used had a pH of about 4.

The oxygen supply was increased by moistening the cellucotton with a commercial solution (about 3%) of hydrogen peroxide. Thirty cc. bottles were used, and the cork was paraffined in place. The hydrogen peroxide was renewed once a week.

The 25°C. temperature was fairly constant, but fluctuated occasionally between 22° and 28°C. The 20°C. likewise fluctuated, although infrequently, between 18° and 22°C. The high temperature, called 30°C. was not controlled, being anywhere between 26°and 31° C. The 5°C. temperature was procured by an electric refrigerator, the fluctuations being from 2° to 5° C. The room in the greenhouse that was used for the soil tests had a range of temperature from 7.5° to 12.5° C. in winter and from 26.5° to 33° C. in the summer.

The number of seeds used varied with their abundance. It will therefore be given for each test. It is rather low in some cases, but the agreement of the results of similar tests shows that no serious error is involved.

## RESULTS

The results are given in the following tables. Table I shows the results for seeds less than one year old, giving a germination of 50% within 365 days. The last column of the table gives the number of days the test was run. In the low temperature tests, this includes the period at 5°C. as well as that at 25° C.

As shown by Table I, the actual percentages for *Hibiscus militaris* Cav. and *Lilium philadelphicum andinum* (Nutt.) Ker. are rather low. Investigating the condition of the embryo of seeds from the same lot, it was found in the case of *Hibiscus*, that of a sample of ten apparently good seeds, six had portions of the embryo decayed. Of another sample of fifteen, ten were similarly decayed. Forty per cent was therefore taken as the probable percentage of sound seed of the lot. In *Lilium* the seeds that did

not germinate did not have embryos.

Lupinus perennis L. and Trillium recurvatum Beck. both show rapid germination of fresh seeds as compared with a much slower germination after storage. This is also seen in the low temperature tests on Trillium recurvatum. In this species, the low temperature does not hasten the germination of either fresh or stored seed. The coat does not bring about a delay since there is no germination when it is scratched. It is permeable to water, and increasing the oxygen supply does not increase the germination.

In Lupinus perennis, however, the complete and rapid germination of stored seeds when the coat is removed shows that the delay is due to the coat. Evidently not during the ripening, as is usual with legumes, but with subsequent dry storage, the outer coat becomes hard and relatively impermeable to water. It gradually regains its permeability after months on a moist substrate.

Hibiscus militaris Cav. shows a slight increase in germination with storage. In Table II, Heuchera hispida Pursh. very markedly has an increased germinative capacity after longer periods of storage. Physostegia virginiana (L.) Benth. may also be of this type, but time may have been a limiting factor in the first test.

Table III gives the results obtained with those species whose germination is increased when the moist seeds are subjected to a few weeks at 5° C. before they are put to 25° C. Of course only those tests are directly comparable that were made with seeds of the same period of storage.

Neither Celtis occidentalis pumila Muhl. nor Pinguicula vulgaris L. give any germination after 180 and 301 days respectively. In both cases, the low temperature brings about germination within a shorter period. In the case of Celtis, the lack

TABLE I

SEEDS GIVING GOOD GERMINATION AT 25° C.

| Name of Seed | Treatment | Days Storage | Per Cent Germination | Corrected Per Cent Germination | Days | Number of Seeds | Total Days for Test |
|---|---|---|---|---|---|---|---|
| Lilium philadelphicum andinum | * | 107 | 10<br>20<br>30<br>40 | 20<br>40<br>60<br>80 | 10<br>31<br>37<br>41 | 10 | 48 |
| Trillium recurvatum | | 1<br>155 | 100<br>10<br>30 | | 38<br>89<br>117 | 10<br>10 | 38<br>117 |
| | 56 days at 5° C. | 1 | 100 | | 90 | 10 | 90 |
| | 84 days at 5° C. | 155 | 0 | | 33 | 20 | 33 |
| | Hydrogen peroxide 3% | 155 | 0 | | 150 | 4 | 150 |
| | Coats Scratched | 315 | 0 | | 18 | 9 | 18 |
| Iris versicolor | | 27 | 12.5<br>25<br>50<br>75<br>87.5 | | 44<br>86<br>105<br>167<br>176 | 8 | 224 |
| Iodanthes pinnatifidus | | 9 | 33<br>93 | | 101<br>117 | 15 | 270 |

*Unless otherwise stated, Petri dishes at 25° C. were used.

TABLE I CONTINUED

| Name of Seed | Treatment | Days Storage | Per Cent Germination | Corrected Per Cent Germination | Days | Number of Seeds | Total Days for Test |
|---|---|---|---|---|---|---|---|
| Lupinus perennis | | 1 | 80 | | 1 | 5 | 3 |
| | | | 100 | | 3 | | |
| | | 1 | 90 | | 1 | 10 | 11 |
| (spotted seed) | | 1 | 60 | | 1 | 10 | 3 |
| | | | 80 | | 3 | | |
| (spotted seed) | | 1 | 60 | | 1 | 10 | 11 |
| | | | 80 | | 3 | | |
| | | 115 | 30 | | 55 | 10 | 193 |
| | | | 40 | | 60 | | |
| | | | 50 | | 74 | | |
| | | | 70 | | 82 | | |
| | | | 90 | | 135 | | |
| | | 357 | 0 | | 18 | 5 | 18 |
| | Coats removed | 357 | 100 | | | | |
| Hibiscus militaris | | 98 | 20 | 50 | 12 | 10 | 474 |
| | Soil | 193 | 36 | 90 | 10 | 25 | 77 |
| | | 475 | 20 | 50 | 4 | 10 | 95 |
| | | | 30 | 75 | 11 | | |
| | | | 40 | 100 | 20 | | |

TABLE II

SEEDS GIVING FAIR GERMINATION AT 25° C.

| Name of Seed | Days Storage | Per Cent of Germination | Days | Number of Seeds | Total Days for Test |
|---|---|---|---|---|---|
| Trillium declinatum | 159 | 10<br>30 | 335<br>425 | 10 | 474 |
| Heuchera hispida | 98<br>460<br>940 | 0<br>90<br>40<br>90± | 122<br>305<br>20<br>70 | 10<br>10<br>200 | 122*<br>305<br>70 |
| Physostegia virginiana | 60<br>479 | 0<br>30 | 134<br>254 | 10<br>10 | 134*<br>432 |

* Tests made by George (7).

TABLE III

SEEDS WHOSE GERMINATION IS INCREASED BY LOW TEMPERATURE

| Name of Seed | Treatment | Days Storage | Per Cent Germination | Days | Number of Seeds | Total Days for Test |
|---|---|---|---|---|---|---|
| Peltandra virginica | | 3 | 20<br>40<br>90 | 45<br>52<br>106 | 10 | 106 |
| | 70 days at 5°C. | 3 | 100 | 18 | 10 | 88 |
| Maianthemum canadenses | | 93 | 10<br>20 | 53<br>161 | 10 | 575 |
| | | 461 | 10<br>20 | 45<br>201 | 10 | 595 |
| | | 515 | 0 | 90 | 10 | 90 |
| | | 115 | 10<br>20<br>30<br>40<br>60<br>70 | 25<br>39<br>65<br>102<br>198<br>270 | 10 | 349 |
| | 70 days at 5°C. | 115 | 50<br>90 | 28<br>38 | 10 | 309 |
| Celtis occidentalis pumila | | 17 | 0 | 180 | 5 | 180 |
| | 70 days at 5°C. | 17 | 20<br>60 | 5<br>26 | 5 | 180 |
| | 98 days at 5°C. | 17 | 20<br>40-<br>60 | 26<br>36<br>56 | 5 | 180 |
| | Coat scratched | 17 | 0 | 180 | 5 | 180 |

TABLE III CONTINUED

| Name of Seed | Treatment | Days Storage | Per Cent Germination | Days | Number of Seeds | Total Days for Test |
|---|---|---|---|---|---|---|
| Pinguicula vulgaris | | 676 | 0 | 301 | 100 | 301 |
| | 126 days at 5° C. | 676 | 60 | 86 | 5 | 301 |
| | 140 days at 5° C. | 676 | 40 | 14 | 5 | 301 |
| | 201 days at 5° C. | 676 | 40 | 23 | 5 | 301 |

of germination, even when the coat is broken, shows that the delay is not caused by the coat.

Maianthemum canadense Desf. and Peltandra virginica (L.) Kunth. germinated to some extent without the low temperature. With it, better germination was obtained in less time. The time is shorter not only because the germination at 25° C. is quicker, but also because the total period of 5° C. plus that at 25° C. is shorter.

Because of the relationship between the effect of temperature and the amount of moisture present, it seemed desirable to contrast the effect of low temperature on moist seeds with that on dry.

The species used were ones that had previously been shown by George or the writer to be benefited by a period at 5° C. With Dodecatheon Meadia L., Polygonatum biflorum (Walt.) Ell., and Smilacina racemosa (L.) Desf. good germination was obtained only after the low temperature-moist treatment. With Maianthemum canadense Desf. and Arisaema triphyllum (L.) Schott good germination

was obtained at 25° C. but the low temperature brought it about more quickly.

The low temperature-dry is not as effective in hastening after-ripening as is the low temperature-moist. This is shown clearly in every case. Has it any advantage over the constant 25° C.? In Arisaema triphylla there is the advantage of a rapid germination at 25° C. although the total time is not decreased. In Dodecatheon meadia, the percentage germination is as low as that at 25° C., while in Maianthemum and Smilacina it is even less. The comparison cannot be made for Polygonatum because of the difference in storage periods for the seeds of the two tests.

Since the sterilization with the 60 minute soaking that it entails might have an effect on subsequent dry treatment, Arisaema and Smilacina were put to 5° C. without having been sterilized, and Polygonatum was tested with a longer pre-soaking period. In Smilacina, the 60 minute soaking previous to the 5° C. dry treatment necessitated a longer period for the same germination. Likewise in Polygonatum, the twenty-four hours soaking decreased the germination. In Arisaema, however, the 60 minute pre-soaking increased the germination in the low temperature-dry test.

Soaking previous to the low temperature-moist treatment increased the germination of Polygonatum biflorum, the only one on which it was tried.

Since Arisaema shows only a slight hastening by the low temperature-moist treatment, it can be considered to be of a different type from the rest. The others show agreement in that the low temperature-dry condition does not hasten after-ripening; soaking the seeds before the low temperature-moist test increases their germination whereas soaking before the low temperature-dry decreases it.

It is of interest to know whether or not the coat plays a part in these results. Miss George tried sulphuric acid on Arisaema, Dodecatheon and Polygonatum but with no success. Scratching the coat and increasing the oxygen sullpy of Polygonatum likewise gave no beneficial results. It appears, therefore, that the coat is not a limiting factor in the germination of Polygonatum, Arisaema and Dodecatheon. The coats of Maianthemum and Smilacina are similar to that of Polygonatum.

Table V shows the beneficial effect of scratching the coat of seeds of Baptisia leucantha T. and G. Without this, no germination was obtained even after 477 days, but with it, 80% in one case and 75% in another case occurred in a much shorter time. There was not complete germination because the other seeds molded. The coat is impermeable to water.

TABLE V

GERMINATION TESTS ON BAPTISIA LEUCANTHA T. AND G.

| Treatment | Days Storage | Per Cent of Germination | Days | Number of Seeds | Total Days for Test |
|---|---|---|---|---|---|
| | 214 | 0 | 477 | 10 | 477 |
| Coat scratched | 214 | 40<br>80 | 48<br>189 | 5 | 189 |
| Coat scratched | 224 | 75 | 99 | 8 | 99 |
| Hydrogen peroxide 3% | 266 | 0 | 240 | 5 | 240 |

The several tests made by Miss George on Sanguinaria canadensis L. gave no germination except for one seed. She noted, however, that some germinated in nature in one and one-half or two

and one-half years after their formation. In continuing these and other tests, the writer obtained some results that are seemingly significant. These are given in Table VI.

The only cases of germination were in tests of the following treatments: boiling water three minutes, soil, 5° C., coats removed and 5° C., although in several other cases the outer coats were split open.

The period of storage must be taken into account in a comparison of results because it affects the subsequent time before germination. This can be seen from the three-minute boiling water tests where a difference of 372 days storage made a difference of 416 days in the germination. It is probably more nearly correct therefore to use the total time in noting results.

The lack of germination in the several tests at 25° C. with the splitting of the coat of only one seed 1400 days after seed dispersal indicates a dormant period of over 1400 days. The removal of the coat caused a few seeds to germinate in about 600 days after collection. Further germination of these will probably occur later. Evidently there is an embryo dormancy of at least 600 days. Dipping the seeds for three minutes in boiling water brought about 60 to 80% germination in 600-1400 days. Since none occurred before 600 days, the inference is that this treatment affected only the coat.

The low temperature does not hasten the after-ripening, for the only germination of decorticated seeds thus treated occurred after 700 days and with the coats intact, after 1300 days.

Soil as a substrate decreases the embryo dormancy since some germination occurred in 186 and 253 days. Other germination, after 900 days, also occurred, but this could be due to the soil affecting only the coat. The fact that the seeds were put to

TABLE VI

TESTS ON SANGUINARIA CANADENSIS
SHOWING SOME GERMINATION

| Treatment | Days Storage | Per Cent Germination | Days | Number of Seeds | Total Days for Test |
|---|---|---|---|---|---|
| | 193 | 0<br>10% split | 1224<br>1224 | 10 | 1224 |
| Outer coats removed, inner scratched | 254 | 20 | 376 | 5 | 444 |
| Outer coats removed, inner whole | 254 | 20 | 376 | 5 | 444 |
| Boiling water 3 minutes | 493 | 60<br>80<br>100% split | 439<br>922<br>235 | 5 | 922 |
| Boiling water 3 minutes | 121 | 20<br>40<br>60 | 480<br>545<br>855 | 5 | 897 |
| Ether 3 minutes | 963 | 0<br>20% split | 452<br>53 | 5 | 452 |
| Soil, not sterile | 1 | 3.3<br>6.6 | 186<br>253 | 30 | 698 |
| Soil, sterile | 3 | 4 | 467 | 25 | 1032 |
| Soil, outdoors overwinter | 166 | 10 | 730 | 50 | 730 |
| 5° C. 14 days | 196 | 0<br>100% split | 665<br>515 | 5 | 679 |
| 5°C. 21 days | 196 | 0<br>100% split | 658 | 5 | 679 |

TABLE VI CONTINUED

| Treatment | Days Storage | Per Cent Germination | Days | Number of Seeds | Total Days for Test |
|---|---|---|---|---|---|
| 5° C. 28 days | 196 | 0<br>40% split | 651<br>501 | 5 | 679 |
| 5° C. 35 days | 196 | 0<br>40% split | 644<br>494 | 5 | 679 |
| 5° C. 42 days | 196 | 0<br>40% split | 1172<br>487 | 5 | 1215 |
| 5° C. 49 days | 196 | 0<br>40% split | 630<br>480 | 5 | 679 |
| 5° C. 56 days | 196 | 20<br>60% split | 1125<br>473 | 5 | 1215 |
| 5° C. 63 days | 196 | 0<br>40% split | 616<br>466 | 5 | 679 |
| 5° C. 70 days | 196 | 0<br>60% split | 609<br>459 | 5 | 679 |
| 5° C. 84 days | 196 | 0<br>100% split | 594<br>445 | 5 | 679 |
| 5° C. 91 days | 196 | 0<br>40% split | 1124<br>438 | 5 | 1215 |
| 5° C. 98 days | 196 | 0<br>80% split | 581<br>431 | 5 | 679 |
| 5° C. 42 days | 5 | 0<br>60% split | 988<br>988 | 5 | 1050 |
| 5° C. 84 days | 5 | 0<br>60% split | 946<br>946 | 5 | 1050 |
| Coats removed, 5° C. 14 days | 227 | 20 | 482 | 5 | 644 |

TABLE VI CONTINUED

| Treatment | Days Storage | Per Cent Germination | Days | Number of Seeds | Total Days for Test |
|---|---|---|---|---|---|
| Dichlor ethylene vapor, 24 hours | 264 | 0 | 633 | 10 | 633 |
| | | 10 % split | 487 | | |
| Trichlor ethylene vapor, 24 hours | 264 | 0 | 633 | 10 | 633 |
| | | 20 % split | 487 | | |
| Xylol vapor, 24 hours | 264 | 0 | 633 | 10 | 633 |
| | | 70 % split | | | |

germinating conditions directly after collection, does not explain their comparatively rapid germination because others of a similar period of storage which were not in soil did not germinate even after 1000 days.

Soaking the seeds in ether for three minutes has little if any effect on the coat. None of the seeds so treated germinated in less than 1400 days although one has split the coat, indicating that germination is soon to take place.

No germination was obtained with dichlor ethylene, trichlor ethylene or xylol after a total of 900 days, but the coats had split after 600-700 days, indicating that germination would have taken place about a year later. These results show the same amount of hastening as that procured by the boiling water.

Other tests with different temperatures, alternating temperatures, longer periods of boiling water, acids, acid peat, sugars on decorticated seeds, sand, ethylene chlorhydrin, propy-

lene chlorhydrin, sodium thiocyanate, thiourea and ethylene, calculated to effect either the coat or the embryo or both, gave only negative results.

It might be of interest here to note the nature of the coats. There are three: a thin bronzed outer one, then a dull brown coat of egg-shell consistency and finally a thin membranous one. The funiculus is attached to the outer coat all along the back of the seed so that in scraping this off, the outer coat is broken but the middle coat is intact. Since the removal of the funiculus made no difference in the germination except to decrease the mold, the outer coat plays no part in the delay. The tests with the inner coat whole and with it broken showed no difference in germination. The middle layer must, therefore, be the limiting one.

Seeds were soaked and weighed. The water intake was measured as increase in percentage dry weight.

The middle coat is permeable to water, although slowly as compared with the inner one. The presence of the highly absorbent funiculus probably causes the high percentage with coats intact. Increasing the oxygen directly and by means of hydrogen peroxide brought about no germination, so the dormancy is not due to a limiting supply. The remaining possibility as to the effect of the coat is that it offers mechanical restraint to the slowly germinating embryo. This will have to be tested either by breaking the coats after different intervals and noting the time then needed before germination, or by breaking the coat and waxing the crack.

The embryo of stored seed is small although the cotyledons are differentiated. It is one-half mm. long while the seed itself is about 3 mm. After 800 days in germinating conditions it is not

much larger, being 3/4 to 1¼ mm. long, the increase being due to the elongation of the hypocotyl. Between this period and about 1000 cays, there is a comparatively rapid increase, especially in the cotyledons. It is then 2 to 3 mm. long and the coat is split. When it is slightly more than 3 mm. it fills the length of the seed and the hypocotyl starts coming through the broken end of the coat thus "germinating." Even at this stage, there is quite a bit of endosperm along the sides of the embryo. One of the embryos exined had three cotyledons.

Although numerous tests of Hepatica acutiloba D. C. were made under various conditions, the only germination was with seeds put into soil immediately after collection. This germination of 60% occurred after 175 days. In three other tests, germination is about to take place; the seeds are swollen and have burst the fruit coat. The seeds were used 21 days after collection but they were kept in soil in tightly corked bottles during this period of storage so that they would not dry out. One case was the ordinary test; one out of ten seeds splitting after 565 days. The other two were low temperature tests at 5° C. 151 and 175 days respectively. In the former, one of five seeds split in 534 days at the subsequent 25° C. Two out of three of the latter burst their coats after 527 days at 25° C.

Only these seeds with short storage give signs of germination. With longer periods of dry storage they gave no results after 500 days in soil. Either the seeds dry out and die or they acquire a longer dormancy. If it is the latter, the low temperature is not effective in hastening it; nor is the removal of the coat, increased oxygen, ethylene, acid substrate, or sugar on decorticated seeds.

The condition of the embryo makes the former conclusion the more probable. In the stored seed, it is a tiny sphere of undifferentiated cells lying at the base of the endosperm. It is one-half mm. in diameter, while the seed without the coat is 2 x 1 mm. In the seeds about the germinate the embryo is visibly larger, being $1\frac{3}{4}$ x $\frac{1}{2}$ mm. and is differentiated into hypocotyl and cotyledons. No cases are known of such undeveloped embryos acquiring a dormancy. It is more probable that when their continued development is stopped by dry storage death ensues. The 216 days in soil or the 565 days in the Petri dishes are evidently necessary for the development of the embryo.

What factor in the use of soil as a substrate brings about a quicker development than the conditions in the Petri dishes? The apparent difference in this case was in the light. The dishes were kept in the dark germinators while the seeds on the surface of the soil were exposed to light. Testing this possibility by leaving seeds in the light proved unsuccessful. The acidity has often been found to be the factor in the substrate that hastens germination. The soil used in these tests, however, gave a neutral reaction. The temperature of the soil and Petri dish experiments did not differ enough to be significant.

The effect of the soil might be on the coats. There are two coats: an outer hairy fruit coat and an inner thin seed coat. Tests were made with the coats scratched or removed and with increased oxygen. These gave no germination. The embryos of the scratched seeds were still undeveloped. Since the seeds used had had a period of dry storage, they may have been dead from the start.

The permeability of the coats to water was tested more directly by soaking the achenes in water both with and without

TABLE VIII

TESTS ON HEPATICA ACUTILOBA SHOWING SOME GERMINATION

| Treatment | Days Storage | Per Cent Germination | Days | Number of Seeds | Total Days for Test |
|---|---|---|---|---|---|
| | 21 stored in soil | 0<br>10% split | 617<br>565 | 10 | 617 |
| 5° C. 168 days | " | 0<br>20% split | 534<br>534 | 5 | 702 |
| 5° C. 175 days | " | 0<br>66% split | 527<br>527 | 3 | 702 |
| Soil in doors, not sterile | 1 | 20<br>60 | 175<br>216 | 50 | 732 |

fruit coats. The former gave 2600% increase in weight in twenty-four hours and the latter sixty-six per cent. The large increase with the fruit coats on was due partly to the absorption by the hairs and partly to the fact that three of the twenty achenes were empty of seed and so became filled with water.

The possibilities remain of the soil increasing the permeability of either coat to oxygen or of furnishing nutrients that hasten the development of the embryo. These need to be tested when fresh seeds are again available.

The germination of seeds of Sarracenia purpurea L. show interesting results as to the effect of the substrate. As seen in Table IX, germination was obtained only in acid peat, not in

TABLE IX

GERMINATION TESTS ON *SARRACENIA PURPUREA*

| Treatment | Days Storage | Per Cent of Germination | Days | Number of Seeds | Total Days for Test |
|---|---|---|---|---|---|
| | 171 | 0 | 455 | 10 | 455 |
| | 171 | 0 | 455 | 10 | 455 |
| Acid peat | 173 | 10<br>20 | 63<br>150 | 10 | 453 |
| Soil | 173 | 0 | 453 | 10 | 453 |
| Soil outdoors overwinter | 433 | 0 | 168 | 50 | 168 |
| Coat scratched | 511 | 0 | 115 | 10 | 115 |
| Coat scratched Acetic acid n/500 | 511 | 0 | 51 | 10 | 51 |
| Coat scratched Acetic acid n/200 | 511 | 0 | 115 | 10 | 115 |
| 5° C. 70 days | 511 | 0 | 115 | 10 | 115 |
| Coat scratched 5° C. 70 days | 511 | 0 | 115 | 10 | 115 |
| Seeds overwintered in the bog 240 days | 2 | 0 | 41 | 10 | 41 |
| Seeds overwintered in the bog, Acid peat | 2 | 32<br>64 | 29<br>36 | 25 | 41 |
| Seeds overwintered in bog, soaked 24 hours, then acid peat | 2 | 72 | 34 | 50 | 41 |
| Seeds overwintered in bog, Coat scratched, Acid peat | 2 | 0 | 41 | 25 | 51 |

neutral soil nor on the cellucotton of the Petri dish tests. The effect of the acid is not altogether on the coats, since even with the coats scratched, germination on cellucotton did not occur.

The germination with seeds stored indoors for several months was low (20%), while with seeds that had overwintered in the bog it was high (72%). The 5 C. tests gave no results whether or not the coat was scratched. Evidently the low temperature increases and hastens the germination in acid peat but is not effective alone. Acetic acid did not bring about germination probably because the proper concentration was not used.

Negative results have been reported by George for Uvularia perfoliata L., Claytonia virginica L., Podophyllum peltatum L., and Mitella diphylla L. Further tests under various conditions have also been unsuccessful on these seeds as well as on Clintonia borealis (Ait.) Raf. The stages after germination have been noted wherever possible.

The germination of Hibiscus militaris is epigean: the cotyledons become active photosynthetically as soon as the seed germinates. In two months the first true leaf becomes of full size. In one year, the leafy plant is about one foot high.

The leaf of Lilium philadelphicum andinum appears soon after germination begins. A year later the plant is about six inches high and has numerous leaves.

In Lupinus perennis, the cotyledons may be green while still within the coat. They are epigean, coming out in about two days. The first true leaf appears in one to two months, two others rather rapidly succeeding it.

Peltandra virginica furnishes an interesting case of later stages of germination. Although fairly good germination was ob-

tained at 25° C. without the previous low temperature, yet there was no further development. They remained with only the roots and the green coleoptile protruding, yet apparently in good condition. Those that had the low temperature continued to develop and in one month after germination the true leaf appeared. Since the embryo is well developed within the seed coat, "germination" does not require much change. Therefore this condition may be somewhat comparable to lack of development without the low temperature.

Dodecatheon Meadia lifts its small cotyledons to the light in less than one month after germination. Many seedlings get to this stage. They are then very susceptible to slight changes of the water content of the air or soil and so do not survive.

Seeds of Hepatica acutiloba germinate the first fall after their formation. The hypocotyl protrudes about one inch from the coat. Throughout the winter they remain in this condition. The following spring the cotyledons are pulled out of the coat and become active photosynthetically. They retain their activity through out that year and even through the following winter. The shoot appears the second spring and that year only one leaf is formed. Miss George reports these early stages. One would naturally expect from the slowness of these stages that many more years would be necessary before the production of flowers. Seedlings brought in and grown in the greenhouse developed at the same rate as those outdoors. The third fall two leaves were formed on one seedling, and the next year another leaf was added. Four years after the formation of the seed or three and one-half years after its germination, this plant with three leaves flowered. Two flowers were formed, one in March and another in April, each accompanied by a new leaf. Fertile achenes were not set.

The narrow thin cotyledons of Sarracenia purpurea function directly after the emergence of the hypocotyl. In a few weeks the first true leaf appears. A rosette of leaves is formed within the next nine months. At that time there are twelve leaves, but they are only two inches long. Since the mature plants have about this number, perhaps one more year will suffice for their growth and for floral development.

Seedlings of Podophyllum peltatum are not numerous. A few were found in early spring with only the hypocotyl out and others with the thick dark green cotyledons above ground. Since other seeds from the same fruit were just germinating, it is probable that the germination took place that spring rather than the preceding fall. In a month the first leaves appeared. Quite contrary to expectations there were two pairs of simple leaves, one pair at right angles to the other. These leaves were eaton off by pill bugs and so the later stages could not be determined. In the field plants can be found, however, with small bilobed leaves one to two inches broad. From the appearance of the rootstock, these are two or three years old. Various stages also occur from these to plants with full-sized several-lobed leaves and large root stocks. The length of this series indicates that the period between germination and flowering requires about six years.

Tradescantia virginiana L. flowered in one year after germination.

## DISCUSSION

References by George to the seeds used in this study have already been noted. Adams (1) obtains an increase in the germination of seeds of Maianthemum canadensis by leaving them outdoors over winter. This agrees with the above results as to the beneficial effects of the low temperature. Fresh seed indoors gave

28% germination in 198 days and 78% in 612 days outdoors. This germination is somewhat lower than that obtained in our 25° C. and low temperature tests.

His results for Celtis occidentalis are quite different from ours for Celtis occidentalis pumila. Seventy-two per cent in 278 days was obtained with fresh seed in soil indoors as compared with 16% outdoors and dry storage increased the germination to 90% in 131 days. In our tests, seeds stored only 17 days gave no germination in 180 days at 25 C. but they gave 60% in 96 days of the low temperature treatment. These results are seen to be contrary as to the effect of the low temperature. Since soil was used in one case but not in the other, the tests are not altogether comparable. Closely related forms may show such a difference as do species of Trillium in George's work and in the present paper. In the case of Celtis, however, we may not be dealing even with a true varietal difference.

Mitchell (17) gives results for Lilium philadelphicum. She obtained 85% in 105 days in the light and the same percent in 91 days in the dark. The storage period is not given. Probably it is only a few months. These results correspond fairly well with those given above for Lilium philadelphicum andinum.

The relationship between the vitality of seed and the time of "maturity" is one that has received much attention because of its economic and scientific significance. Data on the different behavior of seeds in storage and the effect on this behavior of environmental factors such as temperature, moisture, light, etc., has been accumulating for years. Interpretations have been proposed more recently.

In most cultivated plants the situation met with is that seeds rather rapidly lose their vitality even in dry storage,

while among wild plants they may retain their vitality for years. In some cases the germination is even increased by periods of dry storage.

Duvel states that the loss in vitality for peas and onions and some other cultivated plants is not a simple drying out process since seeds that were more moist lost their vitality sooner. A rather rapid respiration is carried on even in air-dry conditions. It is more rapid with more moisture or with a higher temperature. Respiration or other slow chemical changes decrease the energy available for germination (5). Crocker admits that in some cases the respiration may use up the available food but that usually this is not the limiting factor. He gives reasons for believing that the slow change occurring is a coagulation of the protoplasmic proteins as Ewart had previously suggested (3). For seeds that do not withstand drying, the changes may be quite different.

The coats may affect the viability either by keeping the seeds from drying out or by decreasing gaseous exchange and limiting respiration. Lute finds that seeds of alfalfa with impermeable coats remain viable for years. When the coats are made permeable, vitality is soon lost (16).

Changes in the coat during air-dry storage complicate matters by causing differences in the germination that are not due to changes in viability. Thus in the several species of fleshy fruited seeds shown by Adams (1) to give better germination immediately after collection than after dry storage, the decrease in germination may be due to the coat becoming more impermeable on drying, and remaining so for a while after it is again moist. Such is the case of Lupinus perennis.

Trillium recurvatum is of the type that shows an actual decrease of germinative capacity after a period of dry storage.

Hepatica acutiloba loses its vitality completely and rapidly on drying. Gentiana crinita has also been said to be of this type (20).

Either changes in coat characters or a "ripening" of the embryo might cause an increased germination after a period of dry storage. Crocker reports Amaranthus retroflexus to be of the former type. It does not germinate until after one or more months of dry storage. He concludes that the breaking strength of the coat is decreased by drying (3). In Sanguinaria canadensis the increased germinative capacity is not due altogether to the coat because the difference is shown even when the coats are removed. However, there may be an interaction of coat and embryo effects with that on the embryo persisting. The seeds of Heuchera hispida are so small that the effect of the coat is hard to determine. A few species are shown by Adams (1) to be benefited by dry storage. It is not known which of these really need after-ripening.

The advantage of stratification has been shown for so many species that it is a general method of procedure in efforts to germinate refractory seeds. Kinzel (12), Davis and Rose (4), Barton (2), Adams (1) and George (7) report its effectiveness. Some of these workers have not made certain that the beneficial effect is not due to a cracking of the coat. Davis and Rose (4) have shown that it really hastens after-ripening. The various methods used were similar in that the seeds were subjected, while moist, to a low temperature. Davis and Rose found 5° C. to be the most effective temperature for Crataegus.

What is the effect of the low temperature treatment on dry seeds? It can be considered in two ways: first, as a method of hastening germination, and second, as a method of storage. The results reported above show that with seeds that are hastened by

the low temperature-moist treatment, the low temperature-dry is not as effective. In fact, in most cases it gives poorer results than the ordinary 25° C. test. This is in full agreement with the results given by Adams (1). These show further that in only one case did the low temperature-dry give the best germination where low temperature-moist, room temperature-moist and dry storage both at low temperature and at room temperature were used. It would seem that the low temperature-dry treatment is very infrequently of value as a method of hastening germination.

As a method of storage, is the low temperature-dry any better than the ordinary room temperature dry storage? Roberts (21) reports an average increase by the indoor dry storage of about 4% using varieties of garden seeds. However, among his "typical" results one variety gives a ratio of 69-61 in favor of the cold dry method. Adams also states that "the low temperature-dry treatment is of no value as a method of storage" (1) but a few species in the tables show a decided advantage for this method, while there are others giving a slight difference in its favor. In only a few cases, then, is the cold dry storage better than the ordinary room temperature-dry conditions.

Another question might be raised here. Will a lower temperature produce the same effect on these dry seeds that the 5° C. does on the moist, or cannot these results be brought about no matter how low a temperature is used? This idea is contrary to the view that after-ripening is brought about by a limiting of respiration as compared to other physiological processes. According to this theory the 5° C. dry conditions limit the respiration too much and so lowering the temperature further would only give worse results.

Soil as a substrate has been known in certain cases to give better germination than blotting paper, cotton, etc., that supply only moisture. Since the soil is quite complex, one or more of the several factors might be responsible. Ottenwalder (18) found that it was the acid of the soil that was effective. This may have been on coat or embryo of both (6). Bases likewise may effect coat permeability (3) or catalyze digestive processes. Kidd and West (11) find that much carbon dioxide in the soil can bring about secondary dormancy in some seeds. Since Kidd also found that a slight amount may increase germination in certain cases (10) this amount in the soil may be the determining factor.

Organic material in the soil might hasten embryo development. Among the orchids, a fungus usually furnishes this food (13). However, some seeds may obtain the organic material more directly. That they are able to use such an external supply is shown by Ives' work on hastening the embryo development of the holly by a 5% dextrose solution (8). It has also been determined from the respiratory quotient that some fatty seeds fed with a sugar solution respire the latter rather than the former (19). Thus with seeds like Sanguinaria and Hepatica that seem to have a sufficient supply of food, the soil may furnish it in a more readily accessible form and so hasten germination. Lehmann found that seeds of Ranunculus sceleratus which will not germinate with distilled water will give a high percentage when either earth or Knop's solution is used (15).

For those who wish to grow wild flowers from seed, what general considerations can be drawn from this and previous work? Usually native plants show delayed germination and slow maturity as compared with cultivated plants, although many of the former have a rapid germination and development. Because of the slow

development, case must be taken to keep off animal pests by using wire screens, and to keep out weed seeds by using sterile soil. Species differ widely and so the germinative behavior of the particular species desired should be consulted wherever possible. For a general method with seeds that have not been tested it is best to plant the seeds in soil directly after collection and to leave them outdoors overwinter. In some cases vegetative propagation is a much quicker method and can be done without injury to the parent plant. It is hardly necessary to add that the conditions of acidity and sun should be as much as possible like those of the natural habitat.

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